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NEWS 30	Apr 11	Display formats in DGENE enhanced
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NEWS 32	Apr 17	Polymer searching in REGISTRY enhanced
NEWS 33	Apr 21	Indexing from 1947 to 1956 being added to records in CA/CAPLUS
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NEWS 38	May 15	Supporter information for ENCOMPPAT and ENCOMPLIT updated
NEWS 39	May 16	CHEMREACT will be removed from STN
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=> s bifunctional molecule
L1 643 BIFUNCTIONAL MOLECULE

=> s l1 and 5000 daltons
L2 1 L1 AND 5000 DALTONS

=> d l2 cbib abs

L2 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2002:294009 Document No.: PREV200200294009. Synthetic **bifunctional molecules** containing a drug moiety and presenter protein ligand. Briesewitz, Roger (1); Crabtree, Gerald R.; Wandless, Thomas; Ray, Gregory Thomas; Vogel, Kurt William. (1) Mountain View, CA USA. ASSIGNEE: The Board of Trustees of the Leland Stanford Jr. University; The Howard Hughes Medical Institute, Chevy Chase, MD, USA. Patent Info.: US 6372712 April 16, 2002. Official Gazette of the United States Patent and Trademark Office Patents, (Apr. 16, 2002) Vol. 1257, No. 3, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133. Language: English.

AB **Bifunctional molecules** and methods for their use in the production of binary complexes in a host are provided. The **bifunctional molecule** is a conjugate of a drug moiety and a presenter protein ligand. The molecular weight of the

bifunctional molecule is preferably less than about **5000 daltons**, and the drug moiety may have a molecular weight of from about 50 to 2000 daltons. The drug moiety and presenter protein ligand may be covalently linked directly or through a linking group. The drug moiety binds to a drug target such as a protein and the presenter protein ligand binds to a presenter protein that is not the drug target such as extracellular or intracellular protein. Presenter proteins include peptidyl prolyl isomerase (FKBP), Heat Shock Protein 90 (Hsp90), steroid hormone receptors, cytoskeletal proteins, albumin and vitamin receptors. When the presenter protein is FKBP, ligands include FK506, rapamycin and cyclosporin A which may have an introduced functional group such as hydroxyl, amino, carboxyl, aldehyde, carbonate, carbamate, azide, thiol or ester for attaching the drug moiety. In the methods of use, an effective amount of the **bifunctional molecule** is administered to the host. The **bifunctional molecule** binds to the presenter protein to produce a binary complex such that the drug exhibits at least one of improved affinity, specificity or selectivity as compared to the corresponding free drug. The methods and **bifunctional molecules** find use in a variety of therapeutic applications.

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=> s l1 and protein target
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L3            6 L1 AND PROTEIN TARGET

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L4             2 DUP REMOVE L3 (4 DUPLICATES REMOVED)

=> d l4 1-2 cbib abs

L4   ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2003 ACS
2000:332410  Improving protein-ligand interactions..  Wandless, Thomas J.
     (Department of Chemistry, Stanford University, Stanford, CA, 94305-5080,
     USA). Book of Abstracts, 219th ACS National Meeting, San Francisco, CA,
     March 26-30, 2000, ORGN-283. American Chemical Society: Washington, D. C.
     (English) 2000. CODEN: 69CLAC.
AB   One strategy to improve the binding between a small mol. ligand and its
     protein receptor is to increase the surface area at the binding interface.
     Addnl. interactions may contribute, either pos. or neg., to the overall
     free energy of binding. We have developed a potentially general method to
     improve or diminish the binding affinity between a ligand and its
     protein target. The X-ray structures of two different
     small mols. bound to two different protein targets
     were analyzed. Synthetic chem. was used to link the two compds., thus
     creating a bifunctional ligand for both proteins. When tethered with the
     proper geometry, the bifunctional mol. allows both
     protein receptors to bind simultaneously, and addnl. protein-protein
     interactions are created that contribute pos. or neg. to the overall
     stability of the trimeric complex.

L4   ANSWER 2 OF 2     MEDLINE                              DUPLICATE 1
1999162539  Document Number: 99162539.    PubMed ID: 10051576.   Affinity
     modulation of small-molecule ligands by borrowing endogenous protein
     surfaces. Briesewitz R; Ray G T; Wandless T J; Crabtree G R. (Howard
     Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA. )
     PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF
     AMERICA, (1999 Mar 2) 96 (5) 1953-8. Journal code: 7505876. ISSN:
     0027-8424. Pub. country: United States. Language: English.
AB   A general strategy is described for improving the binding properties of
     small-molecule ligands to protein targets. A
     bifunctional molecule is created by chemically linking a
     ligand of interest to another small molecule that binds tightly to a
     second protein. When the ligand of interest is presented to the target
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protein by the second protein, additional protein-protein interactions outside of the ligand-binding sites serve either to increase or decrease the affinity of the binding event. We have applied this approach to an intractable target, the SH2 domain, and demonstrate a 3-fold enhancement over the natural peptide. This approach provides a way to modulate the potency and specificity of biologically active compounds.

=> s l1 and intracellular space
L5 0 L1 AND INTRACELLULAR SPACE

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L6 7 L1 AND "FK506"

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L7 7 DUP REMOVE L6 (0 DUPLICATES REMOVED)

=> d l7 1-7 cbib abs

L7 ANSWER 1 OF 7 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2002:294009 Document No.: PREV200200294009. Synthetic **bifunctional molecules** containing a drug moiety and presenter protein ligand. Briesewitz, Roger (1); Crabtree, Gerald R.; Wandless, Thomas; Ray, Gregory Thomas; Vogel, Kurt William. (1) Mountain View, CA USA. ASSIGNEE: The Board of Trustees of the Leland Stanford Jr. University; The Howard Hughes Medical Institute, Chevy Chase, MD, USA. Patent Info.: US 6372712 April 16, 2002. Official Gazette of the United States Patent and Trademark Office Patents, (Apr. 16, 2002) Vol. 1257, No. 3, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133. Language: English.

AB **Bifunctional molecules** and methods for their use in the production of binary complexes in a host are provided. The **bifunctional molecule** is a conjugate of a drug moiety and a presenter protein ligand. The molecular weight of the **bifunctional molecule** is preferably less than about 5000 daltons, and the drug moiety may have a molecular weight of from about 50 to 2000 daltons. The drug moiety and presenter protein ligand may be covalently linked directly or through a linking group. The drug moiety binds to a drug target such as a protein and the presenter protein ligand binds to a presenter protein that is not the drug target such as extracellular or intracellular protein. Presenter proteins include peptidyl prolyl isomerase (FKBP), Heat Shock Protein 90 (Hsp90), steroid hormone receptors, cytoskeletal proteins, albumin and vitamin receptors. When the presenter protein is FKBP, ligands include **FK506**, rapamycin and cyclosporin A which may have an introduced functional group such as hydroxyl, amino, carboxyl, aldehyde, carbonate, carbamate, azide, thiol or ester for attaching the drug moiety. In the methods of use, an effective amount of the **bifunctional molecule** is administered to the host. The **bifunctional molecule** binds to the presenter protein to produce a binary complex such that the drug exhibits at least one of improved affinity, specificity or selectivity as compared to the corresponding free drug. The methods and **bifunctional molecules** find use in a variety of therapeutic applications.

L7 ANSWER 2 OF 7 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2002:529979 Document No.: PREV200200529979. Detoxification of **bifunctional molecules** by endogenous proteins. Barglow, Katherine T. (1); Lin, Yun-Ming (1); Braun, Patrick D. (1); Wandless,

Thomas J. (1). (1) Department of Chemistry, Stanford University, Stanford, CA, 94305: kbarglow@stanford.edu USA. Abstracts of Papers American Chemical Society, (2002) Vol. 223, No. 1-2, pp. ORGN 373. print. Meeting Info.: 223rd National Meeting of the American Chemical Society Orlando, FL, USA April 07-11, 2002 ISSN: 0065-7727. Language: English.

L7 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2003 ACS

2001:380414 Document No. 134:371812 Targeted **bifunctional molecules** and therapies based thereon. Briesewitz, Roger; Crabtree, Gerald R.; Wandless, Thomas (Board of Trustees of the Leland Stanford Junior University, USA). PCT Int. Appl. WO 2001035978 A1 20010525, 31 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31702 20001117. PRIORITY: US 1999-PV166580 19991119.

AB Targeted **bifunctional mols**. and methods for their use are provided. The subject targeted **bifunctional mols**. are conjugates of a drug moiety and a targeting moiety, where these two moieties are optionally joined by a linking group. The **bifunctional mols**. are further characterized in that they exhibit a modulated biodistribution upon administration to a host as compared to a free drug control. The subject targeted **bifunctional mols**. find use in a variety of therapeutic applications. For example, a **bifunctional mol**. consisting of a drug moiety covalently joined to sulfisoxazole which is extensively bound by albumin, via an inert linking group is formed. When this **bifunctional mol**. enters the human circulation, it is bound by albumin which keeps the drug of interest in the extracellular environment.

L7 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2003 ACS

2001:203206 Cell-selective detoxification by borrowing endogenous proteins. Lin, Yun-Ming; Braun, Patrick D.; Ray, Gregory T.; Wandless, Thomas J. (Department of Chemistry, Stanford University, Stanford, CA, 94305, USA). Abstracts of Papers - American Chemical Society, 221st, ORGN-698 (English) 2001. CODEN: ACSRAL. ISSN: 0065-7727. Publisher: American Chemical Society.

AB Undesired side effects to mammalian cells are often encountered in enzyme inhibitor-based chemotherapy. Potential cell-selective detoxification could be achieved by converting therapeutic agents into **bifunctional mols**. capable of recruiting endogenous proteins unique to mammalian cells as their protecting groups. Covalent attachment of a dihydrofolate reductase (DHFR) inhibitor to a synthetic ligand (SLF) for **FK506** binding proteins (FKBP) affords a **bifunctional mol**. which retains efficacy against DHFR. However, this efficacy is diminished or lost in the presence of FKBP, presumably due to unfavorable protein-protein interactions in the trimeric complex. Borrowing endogenous proteins by **bifunctional mols**. to achieve cell-selective detoxification could provide a novel strategy for drug development. The synthesis and biochem. assay of a **bifunctional mol**. for DHFR and FKBP will be presented.

L7 ANSWER 5 OF 7 SCISEARCH COPYRIGHT 2003 THOMSON ISI

2000:933643 The Genuine Article (R) Number: 380HZ. Mechanistic studies of affinity modulation. Rosen M K; Amos C D; Wandless T J (Reprint). STANFORD UNIV, DEPT CHEM, STANFORD, CA 94305 (Reprint); STANFORD UNIV, DEPT CHEM, STANFORD, CA 94305; MEM SLOAN KETTERING CANC CTR, CELLULAR BIOCHEM & BIOPHYS PROGRAM, NEW YORK, NY 10021. JOURNAL OF THE AMERICAN

CHEMICAL SOCIETY (6 DEC 2000) Vol. 122, No. 48, pp. 11979-11982.
Publisher: AMER CHEMICAL SOC. 1155 16TH ST, NW, WASHINGTON, DC 20036.
ISSN: 0002-7863. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A synthetic ligand for the protein FKBP12 was covalently linked to a peptide ligand (pYEEI) for the Fyn SH2 protein to create a **bifunctional molecule** called SLFpYEEI. This **bifunctional molecule** can simultaneously bind both proteins to form a trimeric complex. When SLFpYEEI is precomplexed with FKBP12, the peptide ligand binds 6-fold more weakly to the Fyn SH2 domain than SLFpYEEI alone. Isotope-edited NMR spectroscopy was used to investigate the molecular basis for the observed reduction in affinity. The results suggest that interactions between the pYEEI peptide and FKBP12 may play a significant role in diminishing the affinity of SLFpYEEI for the Fyn SH2 domain.

L7 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2003 ACS

1999:763899 Document No. 132:15629 **Bifunctional molecules** and therapies based thereon. Briesewitz, Roger; Crabtree, Gerald R.; Wandless, Thomas; Ray, Gregory Thomas; Vogel, Kurt William (The Board of Trustees of the Leland Stanford Junior University, USA). PCT Int. Appl. WO 9961055 A1 19991202, 67 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US11296 19990521. PRIORITY: US 1998-86451 19980522.

AB Non-naturally occurring bifunctional conjugates Z-L-X (Z = ligand that binds to a specific presenter protein; X = drug moiety; L = optional linker) are provided such that upon entering a cell, Z can bind to its receptor protein (if present) and the effectiveness of X is thereby enhanced or inhibited, depending on the nature of the receptor for Z. Thus, a bifunctional peptide (I) was prepd. which contained **FK506** coupled to phosphotyrosyl-glutamyl-glutamyl-isoleucine (pYEEI), which binds to the SH2 domains of tyrosine kinases Fyn and Lck and to the N-terminal SH2 domain of phospholipase C.gamma. (PLC.gamma.). In the presence of **FK506**-binding protein 52 (FKBP52), I bound the Fyn SH2 domain with 3-fold increased affinity. This effect was reversed by **FK506**, and was not mimicked by FKBP12 despite the similar structure of its binding domain to that of FKBP52; the increase in affinity with FKBP52 was presumably based on favorable protein-protein interactions between the Fyn SH2 domain and FKBP52. On the other hand, formation of a FKBP12-I complex reduced the affinity of I for the PLC.gamma. SH2 domain but not for the Fyn or Lek SH2 domains, suggesting that formation of a binary complex may lead to unfavorable protein-protein interactions between the presenter protein and some targets but not other targets of the drug; therefore, formation of a complex between a **bifunctional mol**. and a presenter protein can be used to create specificity. The cell selectivity of a bifunctional conjugate may be enhanced if the formation of a binary complex reduces binding of the drug to all of its targets in a cell that contains the presenter mol.; if an organism has cells that contain the presenter protein and other cells that do not, the cells lacking the presenter protein will be more affected by the bifunctional conjugate than cells expressing the presenter. Similarly, conjugation of penicillamine (an alk. phosphatase inhibitor) to p-aminosalicylic acid (a ligand for albumin) via glycine modulated the inhibitory activity of penicillamine toward 4 isoforms of alk. phosphatase in the presence of 100 .mu.M serum albumin, but not toward 8 other isoforms.

L7 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2003 ACS

1999:180895 Document No. 130:346784 Affinity modulation of small-molecule

ligands by borrowing endogenous protein surfaces. Briesewitz, Roger; Ray, Gregory T.; Wandless, Thomas J.; Crabtree, Gerald R. (Howard Hughes Medical Institute, Stanford University, Stanford, CA, 94305, USA). Proceedings of the National Academy of Sciences of the United States of America, 96(5), 1953-1958 (English) 1999. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.

AB A general strategy is described for improving the binding properties of small-mol. ligands to protein targets. A **bifunctional mol.** is created by chem. linking a ligand of interest to another small mol. that binds tightly to a second protein. When the ligand of interest is presented to the target protein by the second protein, addnl. protein-protein interactions outside of the ligand-binding sites serve either to increase or decrease the affinity of the binding event. We have applied this approach to an intractable target, the SH2 domain, and demonstrate a 3-fold enhancement over the natural peptide. This approach provides a way to modulate the potency and specificity of biol. active compds.

=> s l1 and cyclosporin A
L8 1 L1 AND CYCLOSPORIN A

=> d l8 cbib abs

L8 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2002:294009 Document No.: PREV200200294009. Synthetic **bifunctional molecules** containing a drug moiety and presenter protein ligand. Briesewitz, Roger (1); Crabtree, Gerald R.; Wandless, Thomas; Ray, Gregory Thomas; Vogel, Kurt William. (1) Mountain View, CA USA. ASSIGNEE: The Board of Trustees of the Leland Stanford Jr. University; The Howard Hughes Medical Institute, Chevy Chase, MD, USA. Patent Info.: US 6372712 April 16, 2002. Official Gazette of the United States Patent and Trademark Office Patents, (Apr. 16, 2002) Vol. 1257, No. 3, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133. Language: English.

AB **Bifunctional molecules** and methods for their use in the production of binary complexes in a host are provided. The **bifunctional molecule** is a conjugate of a drug moiety and a presenter protein ligand. The molecular weight of the **bifunctional molecule** is preferably less than about 5000 daltons, and the drug moiety may have a molecular weight of from about 50 to 2000 daltons. The drug moiety and presenter protein ligand may be covalently linked directly or through a linking group. The drug moiety binds to a drug target such as a protein and the presenter protein ligand binds to a presenter protein that is not the drug target such as extracellular or intracellular protein. Presenter proteins include peptidyl prolyl isomerase (FKBP), Heat Shock Protein 90 (Hsp90), steroid hormone receptors, cytoskeletal proteins, albumin and vitamin receptors. When the presenter protein is FKBP, ligands include FK506, rapamycin and **cyclosporin A** which may have an introduced functional group such as hydroxyl, amino, carboxyl, aldehyde, carbonate, carbamate, azide, thiol or ester for attaching the drug moiety. In the methods of use, an effective amount of the **bifunctional molecule** is administered to the host. The **bifunctional molecule** binds to the presenter protein to produce a binary complex such that the drug exhibits at least one of improved affinity, specificity or selectivity as compared to the corresponding free drug. The methods and **bifunctional molecules** find use in a variety of therapeutic applications.

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nested terms that are not separated by a logical operator.

=> s l1 and tacrolimus
L9 1 L1 AND TACROLIMUS

=> d l9 cbib abs

L9 ANSWER 1 OF 1 MEDLINE
1999162539 Document Number: 99162539. PubMed ID: 10051576. Affinity modulation of small-molecule ligands by borrowing endogenous protein surfaces. Briesewitz R; Ray G T; Wandless T J; Crabtree G R. (Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Mar 2) 96 (5) 1953-8. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB A general strategy is described for improving the binding properties of small-molecule ligands to protein targets. A **bifunctional molecule** is created by chemically linking a ligand of interest to another small molecule that binds tightly to a second protein. When the ligand of interest is presented to the target protein by the second protein, additional protein-protein interactions outside of the ligand-binding sites serve either to increase or decrease the affinity of the binding event. We have applied this approach to an intractable target, the SH2 domain, and demonstrate a 3-fold enhancement over the natural peptide. This approach provides a way to modulate the potency and specificity of biologically active compounds.

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L10 333 DUP REMOVE L1 (310 DUPLICATES REMOVED)

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L10 ANSWER 1 OF 333 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
2003:227262 Document No.: PREV200300227262. Miniaturized cell array methods and apparatus for cell-based screening. Kapur, Ravi (1); Adams, Terri. (1) Gibsonia, PA, USA USA. ASSIGNEE: Cellomics, Inc.. Patent Info.: US 6548263 April 15, 2003. Official Gazette of the United States Patent and Trademark Office Patents, (Apr. 15 2003) Vol. 1269, No. 3, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133. Language: English.

L10 ANSWER 2 OF 333 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2003:248778 Document No.: PREV200300248778. Preparation and use of **bifunctional molecules** having DNA sequence binding specificity. Dervan, Peter B.. ASSIGNEE: California Institute of Technology. Patent Info.: US 6555692 April 29, 2003. Official Gazette of the United States Patent and Trademark Office Patents, (Apr. 29 2003) Vol. 1269, No. 5, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html . e-file. ISSN: 0098-1133. Language: English.

L10 ANSWER 3 OF 333 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2003:98218 Document No.: PREV200300098218. Preparation and use of **bifunctional molecules** having DNA sequence binding specificity. Dervan, Peter B.. ASSIGNEE: California Institute of Technology. Patent Info.: US 6506906 January 14, 2003. Official Gazette of the United States Patent and Trademark Office Patents, (Jan. 14 2003) Vol. 1266, No. 2, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html . e-file. ISSN: 0098-1133. Language: English.

L10 ANSWER 4 OF 333 CAPLUS COPYRIGHT 2003 ACS
2003:242366 Document No. 138:250087 Multiple antigen peptides (MAP) as antidotes against snake neurotoxin intoxication. Bracci, Luisa; Lozzi, Luisa; Lelli, Barbara; Pini, Alessandro; Neri, Paolo (Universita' Degli

Studi di Siena, Italy). PCT Int. Appl. WO 2003025001 A1 20030327, 39 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-IT580 20020912. PRIORITY: IT 2001-RM563 20010914.

L10 ANSWER 5 OF 333 CAPLUS COPYRIGHT 2003 ACS

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71142152 Document Number: 71142152. PubMed ID: 5102346. Studies of organosulfur compounds. I. Reaction between active methyl groups and o-substituted **bifunctional molecule** in the presence of sulfur. Hisano T; Koga H. YAKUGAKU ZASSHI. JOURNAL OF THE PHARMACEUTICAL SOCIETY OF JAPAN, (1971 Feb) 91 (2) 180-6. Journal code: 0413613. ISSN: 0031-6903. Pub. country: Japan. Language: Japanese.

L10 ANSWER 316 OF 333 CAPLUS COPYRIGHT 2003 ACS
1973:424389 Document No. 79:24389 Possible experiments on two-dimensional nematics. De Gennes, P. G. (Phys. Solide, Fac. Sci., Orsay, Fr.). Symposia of the Faraday Society, No. 5, 16-25 (English) 1971. CODEN: FSSYA5. ISSN: 0430-0696.

L10 ANSWER 317 OF 333 CAPLUS COPYRIGHT 2003 ACS
1971:417955 Document No. 75:17955 Conjugated bacteriophage-protein products. (Yeda Research and Development Co. Ltd.). Fr. Demande FR 2028475 19701113, 12 pp. (French). CODEN: FRXXBL. APPLICATION: FR 1970-1597 19700116.

L10 ANSWER 318 OF 333 CAPLUS COPYRIGHT 2003 ACS
1969:502303 Document No. 71:102303 Four-center type photopolymerization in the solid state. III. Polymerization of phenylenediacrylic acids and their derivatives. Suzuki, Fusae; Suzuki, Yasuzo; Nakanishi, Hachiro; Hasegawa, Masaki (Text. Res. Inst. Japan. Govt., Yokohama, Japan). Journal of Polymer Science, Polymer Chemistry Edition, 7(8), 2319-32 (English) 1969. CODEN: JPLCAT. ISSN: 0449-296X.

L10 ANSWER 319 OF 333 CAPLUS COPYRIGHT 2003 ACS
1969:413443 Document No. 71:13443 Preparation and properties of isomeric poly(phenylacetylenes). Kern, Roland J. (Monsanto Co., St. Louis, MO, USA). Journal of Polymer Science, Polymer Chemistry Edition, 7(2), 621-31 (English) 1969. CODEN: JPLCAT. ISSN: 0449-296X.

L10 ANSWER 320 OF 333 CAPLUS COPYRIGHT 2003 ACS
1964:469051 Document No. 61:69051 Original Reference No. 61:11964a-e Michael alkylation ring synthesis. Piperidine and pyrrolidine construction. Dolfini, Joseph E.; Dolfini, Dorothy M. (Purdue Univ., Lafayette, IN). Tetrahedron Letters (31-32), 2103-7 (Unavailable) 1964. CODEN: TELEAY. ISSN: 0040-4039.

L10 ANSWER 321 OF 333 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 136
1964:86779 Document No. 60:86779 Original Reference No. 60:15180a-b The adsorption of **bifunctional molecules** on CaF2. Schreiver, G. D. L.; MacMillan, R. F. G. South African Industrial Chemist, 18(11), 222-3 (Unavailable) 1963. CODEN: SAICA8. ISSN: 0370-8225.

L10 ANSWER 322 OF 333 CAPLUS COPYRIGHT 2003 ACS
1962:425740 Document No. 57:25740 Original Reference No. 57:5179h-i,5180a-f Polyfunctional dinitrophenyl haptens as reagents for elicitation of immediate-type allergic skin responses. Parker, Charles W.; Kern, Milton; Eisen, Herman N. (Washington Univ., St. Louis, MO). Journal of Experimental Medicine, 115, 789-801 (Unavailable) 1962. CODEN: JEMEAV. ISSN: 0022-1007.

L10 ANSWER 323 OF 333 CAPLUS COPYRIGHT 2003 ACS
1962:448339 Document No. 57:48339 Original Reference No. 57:9577a-c Polarographic oxidation and reduction of p-nitroaniline at carbon paste

electrodes. Olson, Carter; Lee, H. Y.; Adams, Ralph N. (Univ. of Kansas, Lawrence). Journal of Electroanalytical Chemistry (1959-1966), 2, 396-9 (Unavailable) 1961. CODEN: JEACAX. ISSN: 0368-1874.

L10 ANSWER 324 OF 333 CAPLUS COPYRIGHT 2003 ACS
1959:62134 Document No. 53:62134 Original Reference No. 53:11195d-i Acetylenic ethers. XXIV. Reactivity of acetylenic ethers and thioethers; the dual character of alkylthio groups. Volger, H. C.; Arens, J. F. (Univ. Groningen, Neth.). Recueil des Travaux Chimiques des Pays-Bas et de la Belgique, 77, 1170-88 (English) 1958. CODEN: RTCPB4. ISSN: 0370-7539.

L10 ANSWER 325 OF 333 CAPLUS COPYRIGHT 2003 ACS
1958:101029 Document No. 52:101029 Original Reference No. 52:17790h Hardening of .omicron.-cresol-formaldehyde resins. Schwaner, Karoly Magyar Kem. Lapja, 13, 68-9 (Unavailable) 1958.

L10 ANSWER 326 OF 333 CAPLUS COPYRIGHT 2003 ACS
1957:81741 Document No. 51:81741 Original Reference No. 51:14812f-h Polyester-diisocyanate polyaddition products of high-molecular weight. (Farbenfabriken Bayer A.-G.). GB 768649 19570220 (Unavailable). APPLICATION: GB .

L10 ANSWER 327 OF 333 CAPLUS COPYRIGHT 2003 ACS
1953:64869 Document No. 47:64869 Original Reference No. 47:10976c-e The kinetics of rapid reactions involving pairs of consecutive second-order steps. Higgins, H. G.; Williams, E. J. (Commonwealth Sci. Ind. Research Organization, Melbourne). Australian Journal of Chemistry, 6, 195-206 (Unavailable) 1953. CODEN: AJCHAS. ISSN: 0004-9425.

L10 ANSWER 328 OF 333 CAPLUS COPYRIGHT 2003 ACS
1953:8402 Document No. 47:8402 Original Reference No. 47:1475b-c Kinetic treatment appropriate to rapid reactions involving two consecutive second-order steps. Higgins, H. G.; Williams, E. J. (Commonwealth Sci. & Ind. Research Organization, Melbourne). Australian J. Sci. Research, 5A, 572-6 (Unavailable) 1952.

L10 ANSWER 329 OF 333 CAPLUS COPYRIGHT 2003 ACS
1945:5384 Document No. 39:5384 Original Reference No. 39:838h-i Improving artificial linearly polymerized masses. (Thuringische Zellwolle A.-G.; Zellwolle und Kunstseide-Ring G. m. b. H). BE 446247 19420731 (Unavailable). APPLICATION: BE .

L10 ANSWER 330 OF 333 CAPLUS COPYRIGHT 2003 ACS
1942:8225 Document No. 36:8225 Original Reference No. 36:1332d-e Polyamides. (E. I. du Pont de Nemours & Co.). GB 535141 19410331 (Unavailable). APPLICATION: GB .

L10 ANSWER 331 OF 333 CAPLUS COPYRIGHT 2003 ACS
1941:42331 Document No. 35:42331 Original Reference No. 35:6579g-i,6580a-c Many-membered ring compounds by direct synthesis from two .omega.,.omega.'-**bifunctional molecules**. Adams, Roger; Whitehill, L. N. J. Am. Chem. Soc., 63, 2073-8 (Unavailable) 1941. CODEN: JACSAT. ISSN: 0002-7863.

L10 ANSWER 332 OF 333 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
1997:296286 Document No.: PREV199799595489. Design and expression of a stable bispecific scFv dimer with affinity for both glycophorin and N9 neuraminidase. Atwell, John L. (1); Pearce, Lesley A.; Lah, Maria; Gruen, L. Clem; Kortt, Alexander A.; Hudson, Peter J.. (1) CSIRO, Div. Biomolecular Eng., 343 Royal Parade, Parkville, 3052 VIC Australia. Molecular Immunology, Vol. 33, No. 17-18, pp. 1301-1312. ISSN: 0161-5890. Language: English.

L10 ANSWER 333 OF 333 CAPLUS COPYRIGHT 2003 ACS

2003:421332 A **Bifunctional Molecule** That Displays Context-Dependent Cellular Activity. Braun, Patrick D.; Barglow, Catherine T.; Lin, Yun-Ming; Akompong, Thomas; Briesewitz, Roger; Ray, Gregory T.; Haldar, Kasturi; Wandless, Thomas J. (Department of Chemistry and the Howard Hughes Medical, Institute Stanford University, Stanford, CA, 94305, USA). Journal of the American Chemical Society ACS ASAP (English). CODEN: JACSAT. ISSN: 0002-7863. Publisher: American Chemical Society.

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L13 45 L11 AND TARGETING

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L15 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2003 ACS
2002:165031 Document No. 136:195367 NF-AT (nuclear factor, activated T-cell) cytosolic and nuclear polypeptides and their use in screening for immunosuppressive agents. **Crabtree, Gerald R.**; Northrop, Jeffrey P.; Ho, Steffan N.; Flanagan, William M. (The Board of Trustees of the Leland Stanford Junior University, USA). U.S. US 6352830 B1 20020305, 83 pp., Cont.-in-part of U. S. 5,989,810. (English). CODEN: USXXAM. APPLICATION: US 1999-232346 19990115. PRIORITY: US 1991-749385 19910822; US 1993-124981 19930920; US 1994-228944 19940418; US 1994-260174 19940613; US 1995-507032 19950731.

AB The invention provides novel polypeptides which are assocd. with the transcription complex NF-AT, and polynucleotides encoding such polypeptides. Specifically, NF-ATc (cytoplasmic) and NF-ATn (nuclear) proteins assoc. in a complex that interacts with an NF-AT DNA binding sequence which may be studied using gel mobility shift assays. The NF-ATc is translocated to the nucleus by an immunosuppressive agent. Immunosuppressive agents may be identified by contacting a NF-ATc protein with a test agent and detg. the level of said complex formation. Furthermore, the NF-ATc or NF-ATn proteins may be immobilized. An NF-AT regulated enhancer region may be linked to a nucleic acid that encodes a protein essential for cell proliferation of viability to assay for nuclear translocation of NF-ATc. Also provided are antibodies to NF-AT polypeptides, polynucleotide hybridization probes and PCR amplification probes for detecting polynucleotides which encode such polypeptides. Transgenes which encode such polypeptides, homologous **targeting** constructs that encode such polypeptides and/or homologously integrate in or near endogenous genes encoding such polypeptides are provided. Non-human transgenic animals which comprise functionally disrupted endogenous genes that normally encode such polypeptides, and transgenic nonhuman animals which comprise transgenes encoding such polypeptides are also provided. Methods for detecting T cells (including activated T cells) in a cellular sample and methods for treating hyperactive or hypoactive T cell conditions are also provided. Methods for screening for immunomodulatory agents, methods for diagnostic staging of lymphocyte differentiation, methods for producing NF-AT proteins for use as research or diagnostic reagents, methods for producing antibodies reactive with the novel polypeptides, and methods for producing transgenic nonhuman animals

are other embodiments of the invention. Methods and agents for activation of NF-AT dependent transcription, including agents which interfere with the prodn., modification of nuclear or cytoplasmic subunits, or the nuclear import of the cytoplasmic subunits are provided. In particular, screening tests for novel immunosuppressants are provided based upon the ability of NF-AT to activate transcription.

L15 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2003 ACS
2001:27418 Document No. 134:96299 NF-AT polypeptides and polynucleotides and method for detection of T cells. **Crabtree, Gerald R.**; Northrop, Jeffrey P.; Ho, Steffan N. (The Board of Trustees of the Leland Stanford Junior University, USA). U.S. US 6171781 B1 20010109, 99 pp., Cont.-in-part of U.S. Ser. No. 260,174. (English). CODEN: USXXAM. APPLICATION: US 1998-49691 19980327. PRIORITY: US 1993-124981 19930920; US 1994-260174 19940613.

AB The invention provides novel polypeptides which are assocd. with the transcription complex NF-AT, polynucleotides encoding such polypeptides, antibodies which are reactive with such polypeptides, polynucleotide hybridization probes and PCR amplification probes for detecting polynucleotides which encode such polypeptides, transgenes which encode such polypeptides, homologous **targeting** constructs that encode such polypeptides and/or homologously integrate in or near endogenous genes encoding such polypeptides, nonhuman transgenic animals which comprise functionally disrupted endogenous genes that normally encode such polypeptides, and transgenic nonhuman animals which comprise transgenes encoding such polypeptides. The invention also provides methods for detecting T cells (including activated T cells) in a cellular sample, methods for treating hyperactive or hypoactive T cell conditions, methods for screening for immunomodulatory agents, methods for diagnostic staging of lymphocyte differentiation, methods for producing NF-AT proteins for use as research or diagnostic reagents, methods for producing antibodies reactive with the novel polypeptides, and methods for producing transgenic nonhuman animals. The proteins and polynucleotides encoding NF-AT and a dominant neg. mutant of NF-AT provide reagents for screening compds. which modulate NF-AT translocation across nuclear membranes, compds. which bind to the NLS (nuclear localization sequences) of NF-AT, and compds. which promote or inhibit phosphorylation of NF-AT.

L15 ANSWER 3 OF 11 MEDLINE
2001385235 Document Number: 21332584. PubMed ID: 11439183. Signals transduced by Ca(2+)/calcineurin and NFATc3/c4 pattern the developing vasculature. Graef I A; Chen F; Chen L; Kuo A; **Crabtree G R.** (Department of Developmental Biology, Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA.) CELL, (2001 Jun 29) 105 (7) 863-75. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.

AB Vascular development requires an orderly exchange of signals between growing vessels and their supporting tissues, but little is known of the **intracellular** signaling pathways underlying this communication. We find that mice with disruptions of both NFATc4 and the related NFATc3 genes die around E11 with generalized defects in vessel assembly as well as excessive and disorganized growth of vessels into the neural tube and somites. Since calcineurin is thought to control nuclear localization of NFATc proteins, we introduced a mutation into the calcineurin B gene that prevents phosphatase activation by Ca(2+) signals. These CnB mutant mice exhibit vascular developmental abnormalities similar to the NFATc3/c4 null mice. We show that calcineurin function is transiently required between E7.5 and E8.5. Hence, early calcineurin/NFAT signaling initiates the later cross-talk between vessels and surrounding tissues that pattern the vasculature.

L15 ANSWER 4 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
2000:439290 Document No.: PREV200000439290. Regulated transcription of targeted genes and other biological events. **Crabtree, Gerald R.**; Schreiber, Stuart L.; Spencer, David M.; **Wandless, Thomas J.**;

Belshaw, Peter; Ho, Steffan N.. ASSIGNEE: Board of Trustees of Leland Stanford Jr. University, Stanford, CA, USA; President and Fellows of Harvard College. Patent Info.: US 6046047 April 04, 2000. Official Gazette of the United States Patent and Trademark Office Patents, (Apr. 4, 2000) Vol. 1233, No. 1, pp. No pagination. e-file. ISSN: 0098-1133. Language: English.

AB Dimerization and oligomerization of proteins are general biological control mechanisms that contribute to the activation of cell membrane receptors, transcription factors, vesicle fusion proteins, and other classes of intra- and extracellular proteins. We have developed a general procedure for the regulated (inducible) dimerization or oligomerization of **intracellular** proteins. In principle, any two target proteins can be induced to associate by treating the cells or organisms that harbor them with cell permeable, synthetic ligands. To illustrate the practice of tis invention, we have induced: (1) the **intracellular** aggregation of the cytoplasmic tail of the zeta chain of the T cell receptor (TCR)-CD3 complex thereby leading to signaling and transcription of a reporter gene, (2) the homodimerization of the cytoplasmic tail of the Fas receptor thereby leading to cell-specific apoptosis (programmed cell death) and (3) the heterodimerization of a DNA-binding domain (Gal4) and a transcription-activation domain (VP16) thereby leading to direct transcription of a reporter gene. Regulated **intracellular** protein association with our cell permeable, synthetic ligands offers new capabilities in biological research and medicine, in particular, in gene therapy. Using gene transfer techniques to introduce our artificial receptors, one can turn on or off the signaling pathways that lead to the overexpression of therapeutic proteins by administering orally active "dimerizers" or "de-dimerizers", respectively. Since cells from different recipients can be configured to have the pathway overexpress different therapeutic proteins for use in a variety of disorders, the dimerizers have the potential to serve as "universal drugs". They can also be viewed as cell permeable, organic replacements for therapeutic antisense agents or for proteins that would otherwise require intravenous injection or **intracellular** expression (e.g., the LDL receptor or the CFTR protein).

L15 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2003 ACS

1998:734993 Document No. 130:1173 Regulated apoptosis by chimeric proteins binding to FK506-type and cyclosporin-type ligands. **Crabtree, Gerald R.**; Schreiber, Stuart L.; Spencer, David M.; **Wandless, Thomas J.**; Belshaw, Peter (President & Fellows of Harvard College, USA; Board of Trustees of Leland Stanford Jr. University). U.S. US 5834266 A 19981110, 104 pp., Cont.-in-part of U.S. Ser. No. 179,143, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1994-292597 19940818. PRIORITY: US 1993-17931 19930212; US 1993-92977 19930716; US 1993-93499 19930716; US 1994-179143 19940107; US 1994-179748 19940107.

AB A general procedure is described for the regulated (inducible) dimerization or oligomerization of **intracellular** proteins and methods and materials are presented for using that procedure to regulatably initiate cell-specific apoptosis (programmed cell death) in genetically engineered cells. The procedure involves chimeric (or fused) proteins, DNA constructs encoding them, and ligand mols. capable of oligomerizing the chimeric proteins. The chimeric proteins contain at least one ligand-binding (or receptor) domain fused to an action domain capable of initiating apoptosis within a cell (e.g., Fas or tumor necrosis factor receptor), and may also contain addnl. domains for (1) the regulatable or constitutive expression of desired genes and (2) **intracellular targeting**. The chimeric proteins are capable of binding to an FK506-type ligand, a cyclosporin A-type ligand, tetracycline, or a steroid ligand. One such chimeric protein is myristoylated CD3/FKBP12 (MZF3E) receptor consisting of (1) a c-src fragment sufficient for myristoylation, (2) the cytoplasmic tail of .zeta. (a component of the B cell receptor), (3) 3 consecutive domains of the FKBP12 immunophilin, and (4) a flu epitope tag; oligomerization/apoptosis is induced by a dimeric deriv. of FK506. Syntheses are reported for the

prepn. of dimeric and "bumped" (contg. steric bulky groups) derivs. of FK506 and cyclosporin A. The overall procedures allows ligand-mediated oligomerization for regulated gene therapy.

L15 ANSWER 6 OF 11 MEDLINE DUPLICATE 1
97431685 Document Number: 97431685. PubMed ID: 9285717. Rapid **targeting** of nuclear proteins to the cytoplasm. Klemm J D; Beals C R; **Crabtree G R.** (Howard Hughes Medical Institute, Department of Developmental Biology, Stanford University School of Medicine, Stanford, California 94305, USA.) CURRENT BIOLOGY, (1997 Sep 1) 7 (9) 638-44. Journal code: 9107782. ISSN: 0960-9822. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: The transcription factor NF-ATc plays a key role in the activation of many early immune response genes and is regulated by subcellular localization. NF-ATc translocates from the cytoplasm to the nucleus in response to a rise in **intracellular** calcium, and immediately returns to the cytoplasm when **intracellular** calcium levels fall. The rapid nuclear exit of NF-ATc is thought to be one mechanism by which cells distinguish between sustained and transient calcium signals. RESULTS: To study the nuclear export of NF-ATc, we have developed a general, non-invasive assay for the identification and study of nuclear export signals (NESs). The NES is defined by its ability to translocate a protein from the nucleus to the cytoplasm when the two are tethered by a membrane-permeable ligand. This procedure has allowed us to identify a NES within NF-ATc that functions in concert with a glycogen synthase kinase-regulated process to direct the rapid nuclear exit of NF-ATc. CONCLUSIONS: The rapid nuclear export of NF-ATc via its NES and a glycogen synthase kinase-regulated event may be an important mechanism for insulating cells from transient spikes in **intracellular** calcium which might otherwise lead to inappropriate activation. The assay we have developed allows the rapid identification of NESs and can be used as a general method for the inducible cytoplasmic export of nuclear proteins.

L15 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2003 ACS
1996:350220 Document No. 125:27701 Regulatable elimination of gene expression, gene product function and engineered host cells, and its application in gene therapy. Brugge, Joan S.; **Crabtree, Gerald R.** (Ariad Gene Therapeutics, Inc., USA). PCT Int. Appl. WO 9606111 A1 19960229, 141 pp. DESIGNATED STATES: W: AU, CA, GB, JP, KR, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US10591 19950818. PRIORITY: US 1994-292595 19940818; US 1994-292596 19940818; US 1994-292597 19940818.

AB Materials and methods are disclosed for regulated obstruction of the expression of a target gene or the biol. effect of its gene product in genetically engineered cells or organisms contg. them. Aspects of the invention are exemplified by recombinant modifications of host cells and their use in vitro and in vivo for the regulatable blockade of expression of a target gene, for interference with the function or effect of a target gene product or for the regulatable elimination of a target gene. Synthesis of oligomer of ligands such as FK506 and cyclosporin A, and regulation of programmed cell death with immunophilin-Fas antigen chimeras were demonstrated.

L15 ANSWER 8 OF 11 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
96161573 EMBASE Document No.: 1996161573. Controlling protein association and subcellular localization with a synthetic ligand that induces heterodimerization of proteins. Belshaw P.J.; Ho S.N.; **Crabtree G.R.**; Schreiber S.L.. Dept. of Pathology/Devtl. Biology, Howard Hughes Medical Institute, Stanford University Sch. of Medicine,Stanford, CA 94305, United States. Proceedings of the National Academy of Sciences of the United States of America 93/10 (4604-4607) 1996. ISSN: 0027-8424. CODEN: PNASA6. Pub. Country: United States. Language: English. Summary Language: English.

AB Extracellular growth and differentiation factors induce changes in gene expression in the nucleus by initiating a series of protein associations

that alter the subcellular localization of **intracellular** signaling proteins. Initial events involve receptor homo- or heterodimerization and subsequent recruitment of cytosolic signaling proteins to the inner leaflet of the plasma membrane. Intermediate events involve the translocation of proteins into the nucleus. Late events involve the recruitment of transcriptional activators to the vicinity of specific genes in the nucleus, resulting in increased gene transcription. The ability to induce signals at each of these three phases of signaling pathways is illustrated by the use of a heterodimeric chemical inducer of dimerization that causes a proximal relationship between two different target proteins.

L15 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2003 ACS

1995:541403 Document No. 122:283855 Regulated apoptosis by chimeric proteins binding to FK506-type and cyclosporin-type ligands. **Crabtree, Gerald R.**; Schreiber, Stuart L.; Spencer, David M.; **Wandless, Thomas J.**; Belshaw, Peter (Board of Trustees of the Leland Stanford Junior University, USA; President and Fellows of Harvard College). PCT Int. Appl. WO 9502684 A1 19950126, 134 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, UZ, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US8008 19940718. PRIORITY: US 1993-93499 19930716; US 1994-179143 19940107; WO 1994-US1617 19940214.

AB A general procedure is described for the regulated (inducible) dimerization or oligomerization of **intracellular** proteins and methods and materials are presented for using that procedure to regulatably initiate cell-specific apoptosis (programmed cell death) in genetically engineered cells. The procedure involves chimeric (or fused) proteins, DNA constructs encoding them, and ligand mols. capable of oligomerizing the chimeric proteins. The chimeric proteins contain at least one ligand-binding (or receptor) domain fused to an action domain capable of initiating apoptosis within a cell, and may also contain addnl. domains for (1) the regulatable or constitutive expression of desired genes and (2) **intracellular targeting**. The chimeric proteins are capable of binding to an FK506-type ligand, a cyclosporin A-type ligand, tetracycline, or a steroid ligand. One such chimeric protein is myristoylated CD3/FKBP12 (MZF3E) receptor consisting of (1) a c-src fragment sufficient for myristoylation, (2) the cytoplasmic tail of .zeta. (a component of the B cell receptor), (3) 3 consecutive domains of the FKBP12 immunophilin, and (4) a flu epitope tag; oligomerization/apoptosis is induced by a dimeric deriv. of FK506. Syntheses are reported for the prepn. of dimeric and "bumped" (contg. steric bulky groups) derivs. of FK506 and cyclosporin A. The overall procedures allows ligand-mediated oligomerization for regulated gene therapy.

L15 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2003 ACS

1995:377088 Document No. 122:153359 Regulated transcription of target genes with dimeric ligands which cause chimeric receptor proteins to oligomerize and induce gene transcription. **Crabtree, Gerald R.**; Schreiber, Stuart L.; Spencer, David M.; **Wandless, Thomas J.**; Belshaw, Peter (Leland Stanford Junior University, USA; Harvard College). PCT Int. Appl. WO 9418317 A1 19940818, 133 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, SK; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US1617 19940214. PRIORITY: US 1993-17931 19930212; US 1993-92977 19930716; US 1994-179748 19940107.

AB A general procedure for regulating (inducing) dimerization or oligomerization of chimeric proteins is presented. The chimeric proteins contain a receptor domain and another protein domain capable of initiating a biol. process. The chimeric proteins can be induced to assoc. by

treating the cells or organisms that harbor them with cell-permeable, synthetic ligands. The dimers/oligomers bind to a transcription control element and stimulate transcription of the gene to which it is assocd. The syntheses of FK-506 dimers are presented. Such dimers were used to induce: (1) the **intracellular** aggregation of the cytoplasmic tail of the zeta chain of the T cell receptor (TCR)-CD3 complex thereby leading to signaling and transcription of a reporter gene, (2) the homodimerization of the cytoplasmic tail of the Fas receptor thereby leading to cell-specific apoptosis (programmed cell death) and (3) the heterodimerization of a DNA-binding domain (Gal4) and a transcription-activation domain (VP16) thereby leading to direct transcription of a reporter gene.

L15 ANSWER 11 OF 11 MEDLINE DUPLICATE 2

90368794 Document Number: 90368794. PubMed ID: 2394747. Cell type specificity and activation requirements for NFAT-1 (nuclear factor of activated T-cells) transcriptional activity determined by a new method using transgenic mice to assay transcriptional activity of an individual nuclear factor. Verweij C L; Guidos C; **Crabtree G R**. (Howard Hughes Medical Institute, Stanford, California.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1990 Sep 15) 265 (26) 15788-95. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Nuclear factor of activated T-cells (NFAT-1) is a transcription factor which is considered to be an important regulator in early T-cell activation. We have developed a system to monitor the transcriptional activity of NFAT-1 at the single cell level in whole animals. The system is based on the use of an oligomerized NFAT-1 binding motif that directs transcription of SV40 T-antigen in transgenic mice. This report represents the first demonstration that a multimerized short binding motif can function appropriately in transgenic mice. NFAT-1 activity had previously been thought to be confined to activated T-lymphocytes upon release of **intracellular** calcium. By **targeting** NFAT-1-dependent gene expression in transgenic mice we discovered new sites of NFAT-1 activity. Besides in T-lymphocytes NFAT-1 activity could also be induced in T-lymphocyte-depleted spleen cells and purified B-lymphocytes and requires agents that both release **intracellular** calcium and activate protein kinase C. A difference in the time course of appearance of NFAT-1 activity between T-lymphocytes and non-T-lymphocytes was revealed. Constitutive expression was observed in a small population of cells in the dermis and some mice have developed skin lesions. Interestingly, the tissue pattern of expression of the NFAT-1 activity resembles the expression pattern described for HIV-LTR/tat transgenic mice (Vogel, J., Hinrichs, S. H., Reynolds, R. K., Luciw, P. A., and Jay, G. (1988) Nature 335, 606-611). This similarity in expression and the fact that NFAT-1 has been shown to bind functional sequences in HIV-LTR suggest a role for NFAT-1 in dermal activation of the HIV-LTR.

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